Neurobiology of the Skin

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Abstracts

Neurotrophins in healthy and diseased skin

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Neurotrophins (NT) exert their functions through two transmembrane receptors, the low-affinity receptor p75 (p75NTR) and the high-affinity receptors trks. NT form a complex network at the skin level involving most cell types and resulting in a number of autocrine and paracrine activities. Basal keratinocytes secrete biologically active NGF, NT-3, BDNF and NT-4. Furthermore, human keratinocytes express p75NTR, trkA and trkC. Autocrine NGF stimulates keratinocyte proliferation through its high affinity receptor trkA, while K252, a specific inhibitor of trk phosphorylation, blocks this effect. In addition, K252 and anti-NGF antibodies, induce apoptosis in human keratinocytes, indicating that autocrine NGF protects these cells from programmed cell death through its high affinity receptor. While NGF, released from keratinocytes, exert neurotropic properties and stimulate dendricity in melanocytes, these cells produce all NT in different amounts. UVB upregulate the release of NT-4 by keratinocytes that can act in a paracrine manner on melanocytes. Melanocytes in turn produce all NT, but release only NT-4. Melanocytes treated with TPA express TrkA and TrkB, but not TrkC. NT fail to stimulate melanocyte proliferation, whereas they stimulate the synthesis of tyrosinase. NT-4, which is the only NT released by melanocytes acts in an autocrine manner to stimulate melanogenesis. Both dermal fibroblasts (DF) and myofibroblasts (MF) synthesize and release all NTs. In addition, DF and MF express all NT receptors except for trkC. NGF, BDNF, NT3 or NT4 promote fibroblasts differentiation into myofibroblasts, as they induce α-sma expression with an effect similar to the one produced by TGF-β Moreover, NGF and BDNF, but not NT-3 and NT-4 stimulate isometric contractile strengths in living dermal equivalents, as measured by the Glasbox device. Furthermore, scratching assay performed on fibroblasts treated with all four NTs, demonstrates that NGF, BDNF or NT3 also induce fibroblasts migration. These data clearly indicate that the NT network in the skin could be responsible for a variety of functions, from interference with UV damage to melanogenesis, from regulation of contractile properties of the dermis to control of epidermal homeostasis.

Skin mast cells and cutaneous sensory nerves – duo infernale of pruritus

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Mast cells (MCs) are key contributors to many chronic inflammatory and pruritic skin conditions such as urticaria and atopic dermatitis that are strongly modulated by neurogenic signals. Interestingly, there is a close relation of skin MCs and cutaneous sensory nerves (SNs) and MCs and SNs share a number of activating signals and mediators, for some of which both cell types express receptors. Interestingly, a number of recent studies have identified and characterized novel and relevant pathways of MC and SN interactions. SNs were found to increase MC activation induced by IgE and allergen, and MC-mediated inflammation as assessed by using mice that had been subjected to denervation by surgical ablation of their cutaneous SNs. Vice versa, capsaicin treatment, which is known to release neuropeptides from SNs, was found to induce markedly reduced skin inflammation in MC-deficient mice, which indicates that MCs contribute to the induction of SN-dependent inflammation. In addition, MCs were found to control the levels of the neuropeptide endothelin-1, which is upregulated in various inflammatory skin conditions. This novel MC function was shown to involve the release of pre-stored MC proteases and this mechanism proved to contribute to the control of endothelin-1-mediated inflammation *in vivo*. Taken together, these observations show that MCs and SNs work as functional units in the induction and termination of pruritic inflammatory skin reactions. The bidirectional interaction of MCs and SNs in the induction and control of pruritus and cutaneous inflammation may provide novel therapeutic targets for the treatment of various chronic inflammatory and pruritic skin diseases.

Neuroreceptors involved in pruriception

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Itch was regarded for a long time as subspecialty of pain. Now it had become clear that pruriception is mediated by specialized peripheral and central neurons. However, nociception and pruriception show overlapping immunoneurological mechanisms and central perception areas. Unmyelinated skin C-fibers of different classes are involved in induction of several itch qualities. Mechano-insensitive C-fibers express the histamine receptor and mediate almost pure itch. Histamine is involved for example in urticaria-induced itching and may be suppressed with antihistamines. Only recently, mechano-responsive polymodal C-fibers were identified to mediate next to pain also itch. These intraepidermal C-fibers were further identified to express several receptors involved in pruriception. For example, the transient receptor potential vanilloid 1 (TRPV1) may induce burning pain and aggravate pruritus. Thus, targeting the receptor by its ligand capsaicin may serve as therapeutic intervention in chronic pain and pruritus. Also endothelin receptors and the proteinase activated- receptor 2 (PAR-2) are expressed on the mechano-responsive polymodal C-fibers and mediate itch. Current investigations disclosed that many of the involved neuroreceptors are co-expressed, interact and potentate their effects. As a consequence, chronification of the symptoms is facilitated. Mediators of inflammatory cells such as bradykinin and prostaglandin further contribute to chronification by affecting neuroreceptors and by converting their threshold. Moreover, nerve growth factor (NGF) has a high impact on neuroreceptors as well as on neuroanatomy and was described to be a major mediator in chronic pruritic diseases such as prurigo nodularis and atopic dermatitis. The opposite, neuroreceptors such as cold receptors (TRPA1, TRPM8), opioid receptor and cannabinoid receptors may interrupt pruriception and therefore represent novel targets for new therapeutical modalities in chronic pruritus.

Alpha-MSH and fragments: mediators of neuroinflammation with a therapeutic potential

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Neuropeptides are now well appreciated to exert cytokine like effects and to function as mediators of immunity and inflammation. Among these neuromediators α-melanocyte-stimulating hormone (α-MSH) derived from the proopiomelanocortin was found to exert potent immuno-regulatory and anti-inflammatory activities. The biologic activities of α-MSH are exerted via direct effects on cells of the immune system as well as indirectly via affecting the function of resident non-immune cells. Most of these effects are mediated via specific melanocortin receptors (MC-R) in particular MC-1R and MC-3R which are expressed on both immunocompetent as well as non-immune cells. α-MSH affects several pathways implicated in regulation of inflammatory responses such as NF-κB activation, expression of adhesion molecules and chemokine receptors, production of proinflammatory cytokines and chemokines. Thus α-MSH modulates the proliferation, activity, and migration of inflammatory cells as well as programmed cell death. Moreover, α-MSH prevents the maturation of dendritic cells (DC) and thereby triggers the generation of a subset of regulatory T-cells. The anti-inflammatory and immuno-modulatory effects of α-MSH have been confirmed in several animal models of inflammation such as irritant and allergic contact dermatitis, cutaneous vasculitis, asthma, inflammatory bowel disease and rheumatoid arthritis. Most of the anti-inflammatory activities of α-MSH can be attributed to its C-terminal tripeptide KPV. K(D)PT, a derivative of KPV corresponding to the amino acid 193-195 of IL-1β, is currently emerging as another tripeptide with potent anti-inflammatory effects. The anti-inflammatory potential together with the favourable physiochemical properties most likely will allow these agents to be developed for the treatment of inflammatory skin, eye and bowel diseases, allergic asthma, and arthritis.

Catecholamines and stress

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Stress, both acute and chronic, can impair cutaneous wound repair, and this has previously been mechanistically ascribed to stress-induced elevations of cortisol. Our work supports an alternate explanation: that the stress-induced hormone epinephrine directly impairs keratinocyte motility and wound re-epithelialization. We have proposed the burn wound as a prototype of a high-stress, high-epinephrine, wound environment. When human keratinocytes are cultured in medium in which epinephrine levels are elevated to the range found in burn-stressed animals, their migratory rate is profoundly decreased. This impairment is reversed by β2AR antagonists, is absent in murine keratinocytes that are genetically depleted of the β2AR. Activation of the β2AR in cultured keratinocytes signals the down-regulation of the AKT pathway, accompanied by a stabilization of the actin cytoskeleton and an increase in focal adhesion formation, resulting in a nonmigratory phenotype. Burn wound injury in excised human skin also rapidly up-regulates the intra-epithelial expression of the epinephrine synthesizing enzyme phenylethanolamine-N-methyltransferase, and tissue levels of epinephrine rise dramatically (15-fold) in the burn wounded tissue. Finally, using an animal burn wound model (20% body surface in mice), we found that systemic treatment with βAR antagonists results in a significant increase in the rate of burn wound re-epithelialization. This work demonstrates an alternate pathway by which stress can impair healing: by stress-induced elevation of epinephrine levels resulting in activation of the keratinocyte β2AR and the impairment of cell motility and wound re-epithelialization. Furthermore, since the burn wound locally generates epinephrine in response to wounding, epinephrine levels are locally, as well as systemically, elevated, and wound healing is impacted by these dual mechanisms. Treatment with beta adrenergic antagonists significantly improves the rate of burn wound re-epithelialization. This work suggests that specific β2AR antagonists may be apt, near-term translational therapeutic targets for enhancing burn wound healing, and may provide a novel, low-cost, safe approach to improving skin wound repair in the stressed individual.

"fMRI brain scan studies in dermatology"

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Functional magnetic resonance imaging (fMRI) of the brain is able to anatomically

map areas responsible for a variety of cerebral functions. The technique is dependent on blood oxygen levels. We have used fMRI to investigate whether the social impact of psoriasis is associated with altered cognitive processing of the expression of disgust. We demonstrated that psoriasis patients have significantly smaller signal responses to disgusted as compared to neutral facial expression in the bilateral insulae as compared with healthy controls. There was no difference between the groups in terms of processing of fearful facial expressions. This would indicate that patients develop a coping mechanism by blocking processing of disgusted facial expressions encountered in others. We have also investigated the cerebral processing of itch using a placebo–controlled fMRI experimental design and a novel time-series analysis technique using either histamine or saline injected into the volar left forearm. During the histamine scan, all volunteers perceived itch and developed a wheal and flare reaction. In contrast in the saline control there was minimal itch, and wheal and flare reactions were not observed. We showed that there was involvement of the bilateral pre-frontal motor cortex superior and mid-temporal gyri, cerebellum, anterior insula and postcentral gyri in itch processing. These studies, taken together, provide further evidence for the presence of a "brain-skin axis".

Psychoneuroimmunology – clinical implications

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Progress in basic science resulted in the discovery of many mechanisms related to neuropeptides in the skin. These substances are released from peripheral cutaneous nerves during the physiological response to various nociceptive stimuli as well as in disease states. Both histamine from mast (MC) cells and endothelin 1 from endothelial cells and mast cells may induce neurogenic inflammation through their specific receptors on cutaneous sensory fibres H1 and ETA respectively. Neurogenic inflammation is altered in both psoriasis and atopic dermatitis (AD). The activation of vanilloid receptor VR1 is related to pruritus/burning and erythema induced during external calcineurin inhibitor treatment in numerous dermatological diseases. Keratinocytes (KC) are the source of nerve growth factor (NGF), that is neurotropin responsible for cutaneous nociception, nerve development and reconstruction of peptinergic C fibres after injury. Other receptors i.e. thermoreceptor on sensory C and filters Aδ fibres, namely VR1 (transient receptor potential, TRPV1) and cold receptors (TRPM8) on myelinated Aδ fibres might be responsible for itching and burning sensation. Endogenous opioid system is involved in antinociceptive pruritogenic effect in the skin after opioid binding to receptor: β-endorphin-MOR (μ), enkephalins – KOR (δ), and dynorphins – KOR (κ). β-endorphin upregulates some functions of keratinocytes – TGFβR2 expression, cytokeratin 16 production and migration of KC. It stimulates proliferation of melanocytes and plays a role in psoriasis, BCC and wound healing. Chronic itch was related rather to MOR, whereas acute itching to the activity of KOR agonists similarly to histamine, prostaglandins, leukotrienes, SP and CGRP, tryptase etc. Important propruritic factor in the pathogenesis of AD are proteinase activation receptors (PAR2) on skin nerves, which are stimulated by tryptase from MC or by other neutral proteinases. New strategy to suppress nociception is N-palmitoylethanolamin (PEA) which bind cannabinoid receptors CB1 and CB2 which is followed by release of opioids to the skin. These complicated network of neuropeptides is modulating both KC function and immunological response in the skin.

ItchyQoL and CU-Q_2oL – novel instruments for measuring quality of life in patients with pruritic skin disorders

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Pruritus is a widely spread symptom in numerous dermatologic and systemic diseases that may have an enormous impact on patients' daily life. The high individual burden of chronic pruritus along with the difficulty in objectively measuring pruritus intensity makes the assessment of quality of life (QoL) a suitable instrument to evaluate the impact of itch and the efficacy of its therapy upon a patient. Chronic spontaneous urticaria (CU) is a common skin disorder which is characterized by intense pruritus and spontaneously arising wheals and/or angioedema, occurring on a regular basis for longer than 6 weeks. Patients often suffer from CU for many years, and it has repeatedly been found that CU has a substantial impact on patient quality-of-life. In general, a better understanding of QoL in pruritus and CU patients is necessary for both clinical research and routine patient care. In clinical research, QoL measurements provide a rich evaluation of the benefits of a treatment, as experienced from the patient's perspective. In routine care, QoL measures can provide physicians with a better understanding of the ways in which they can help their patients. Although there are many excellent general and dermatological QoL questionnaires, there were until recently no questionnaires available specifically designed for pruritus or CU patients which address the aspects of QoL most relevant to their condition. In the last years, ItchyQoL, an instrument for assessing the pruritus-specific QoL, and CU-Q_2oL, a tool to evaluate the impact of CU on QoL have been developed in English and Italian, respectively, and proven to be reliable, valid and responsive. We have translated both original instruments into German, culturally adapted, retranslated and tested them in cooperation with the original authors. Further translations of these questionnaires into various different languages are now of importance as these tools can then be used for the conduction of international studies with pooled data which would allow, for example, for an objective evaluation of the impact of certain treatments on the QoL in large numbers of pruritus or CU patients from different nationalities. Future use of these patient-related outcome measures may improve efficacy of treatment in pruritus and CU patients and generate direct patient benefit.